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PB-284 755

Endrin and Malathion Toxicity to Flagfish (Jordanella floridae)

U.S. Environmental Research Lab -Duluth, Minn

8 Mar 77

TECHNICAL REPORT DATA
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1. REPORT NO. EPA-600/J-78-018	2. JOURNAL ARTICLE	3. PB 284 755
4. TITLE AND SUBTITLE: Endrin and Malathion Toxicity to Flagfish (Jordanella floridae)		5. REPORT DATE
		6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S): Roger O. Hermanutz		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS: U.S. Environmental Protection Agency, Environmental Research Laboratory-Duluth, 6201 Congdon Boulevard, Duluth, Minnesota 55804		10. PROGRAM ELEMENT NO. 1BA608
		11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS: Same		13. TYPE OF REPORT AND PERIOD COVERED
		14. SPONSORING AGENCY CODE EPA-600/03

15. SUPPLEMENTARY NOTES: Reference: Roger O. Hermanutz. 1978. Endrin and Malathion Toxicity to Flagfish (Jordanella floridae). Arch. Environ. Contam. Toxicol. 7, 159-168.

16. ABSTRACT

Endrin and malathion effects on survival, growth, and reproduction of flagfish were determined in a flow-through system. Endrin chronic effects on first-generation growth and reproduction occurred only at 0.3 µg/L, the highest concentration tested. The mean endrin residues in the first-generation fish at 65 days were about 15,000 times the water concentration in all concentrations tested. Malathion concentrations of 31.5 µg/L and 24.7 µg/L, the highest concentrations tested, had detrimental effects on first-generation survival. During the same period malathion concentrations as low as 10.9 µg/L significantly affected growth. Neither insecticide, at the concentrations tested, had any detectable effects on the second generation during a 30-day exposure period.

Acute toxicities to juvenile fish were also determined in the same flow-through system. The 96-hr LC50's were 0.85 µg/L for endrin and 349 µg/L for malathion.

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U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Insecticide Fish Toxicity Endrin Malathion Flagfish	Insecticide toxicity to fish Endrin Malathion toxicity to flagfish	06/T

18. DISTRIBUTION STATEMENT: RELEASE TO PUBLIC	19. SECURITY CLASS (This Report): UNCLASSIFIED	21. NO. OF PAGES: 10 11
	20. SECURITY CLASS (This page): UNCLASSIFIED	22. PRICE: A02-A01

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

EPA-600/J-78-018
JOURNAL ARTICLE

Arch. Environm. Contam. Toxicol. 7, 159–168 (1978)

Archives of Environmental Contamination and Toxicology

Endrin and Malathion Toxicity to Flagfish (*Jordanella floridae*)

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Abstract. Endrin and malathion effects on survival, growth, and reproduction of flagfish were determined in a flow-through system. Endrin chronic effects on first-generation growth and reproduction occurred only at 0.3 μg/L, the highest concentration tested. The mean endrin residues in the first-generation fish at 65 days were about 15,000 times the water concentration in all concentrations tested. Malathion concentrations of 31.5 μg/L and 24.7 μg/L, the highest concentrations tested, had detrimental effects on first-generation survival. During the same period malathion concentrations as low as 10.9 μg/L significantly affected growth. Neither insecticide, at the concentrations tested, had any detectable effects on the second generation during a 30-day exposure period.

Acute toxicities to juvenile fish were also determined in the same flow-through system. The 96-hr LC50's were 0.85 μg/L for endrin and 349 μg/L for malathion.

The purpose of this study was to determine in acute and life-cycle exposures the sensitivity of flagfish (*Jordanella floridae*) to a representative of each of two major insecticide groups; endrin, an organochloride, and malathion, an organophosphate.

Endrin and malathion are used extensively in agriculture, but information on their chronic toxicity to freshwater fishes is limited. Partial life-cycle exposures of bluntnose minnows (*Pimephales notatus*) and guppies (*Lebistes reticulatus*) to endrin were completed by Mount (1962). Johnson (1967) reported endrin effects on the reproduction of medaka (*Oryzias latipes*) during 23- to 45-day exposures. The chronic effects of malathion on fathead minnows (*Pimephales promelas*) (Mount and Stephan 1967) and bluegills (*Lepomis macrochirus*) (Eaton 1970) have been determined in the laboratory through partial life-cycle exposures. No information was found on complete life-cycle exposures of freshwater fishes to either insecticide.

Materials and Methods

Flagfish were exposed to endrin and malathion separately but concurrently in similar side-by-side systems. All testing procedures were the same for both systems except where noted in the following sections.

0090–4341/78/0007–0159 $2.00
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Life-cycle exposures

Physical

Lake Superior water was sterilized with ultraviolet light and delivered to upper and lower duplicate chambers with proportional diluters (Mount and Brungs 1967). The location of each duplicate was determined by stratified randomization. Lower chambers were directly under the upper chambers, and both levels had similar lighting systems. Each 90×30×30 cm spawning chamber contained two 26×13×30 cm larvae chambers and a flow rate of about 8 spawning-chamber volumes per 24 hr was split equally among them. Water from the larvae chambers entered the spawning chamber through a screened end wall, and standpipes in each spawning chamber maintained a 20-cm water depth in all chambers. Water volume was 54 L for spawning chambers and 6.3 L for larvae chambers. Spawning-chamber water was gently aerated with air stones throughout the experiment to maintain a dissolved oxygen concentration above 80% saturation in all chambers. A constant 16-hr photoperiod was maintained throughout the tests. Temperatures in both systems ranged between 23.1° and 26.6° C for all chambers; the mean temperatures of individual chambers ranged between 25.1° and 25.4° C for both systems.

Chemical

Solutions of acetone and the technical grade insecticides (endrin-96.1% pure; malathion-95% pure) were delivered to the dilution water with a Mariotte-bottle metering device (Mount and Brungs 1967). Acetone was proportional to the insecticide concentrations and the amount of acetone added to the high concentrations never exceeded 3.1 mg/L in the endrin and 1.4 mg/L in the malathion systems. No acetone was added to the control water. Toxicant concentrations in the water were measured weekly by gas chromatographic analysis (U.S. Environmental Protection Agency 1974). Duplicate concentrations were alternately analyzed each week. Samples were taken and analyzed in sets and each set contained single samples of the seven concentrations, a control, a reagent blank and a control spiked with a known amount of the insecticide. Mean recovery and standard deviation of the spiked samples for the entire experiment was 100 ± 12% for endrin and 95 ± 9% for malathion. Individual malathion concentration values were adjusted for the 95% recovery.

Values from duplicate concentrations were grouped to obtain one mean for each concentration (Table 1). In the malathion experiment, the two highest concentrations decreased with time (Figure 1) but the five lowest concentrations showed little or no evidence of decreasing trends. Concentrations were below detection limits of 0.001 μg/L for endrin and 0.01 μg/L for malathion in the control water and below 0.01 ppb for endrin and 0.1 ppb for malathion in the food. Water characteristics determined weekly by methods described by the American Public Health Association *et al.* (1971) were as follows: pH, 7.3–7.6; acidity, 1.7–4.6 mg/L; alkalinity, 39–44 mg/L (as $CaCO_3$); and total hardness, 41–46 mg/L.

Biological

Forty 1- to 2-day-old larvae, produced by laboratory-cultured fish, were randomly placed into one of the larvae chambers in each of the spawning chambers on January 9, 1974. Growth and survival were determined on the 30th exposure day by a photographic method (McKim and Benoit 1971) and a random sample of 15 fish were then transferred to their respective spawning chambers. Survival was also determined during 30- to 65-day and 65- to 110-day exposure periods.

During the 6th week the number of fish was reduced to two males and five females by random

Table 1. Measured endrin and malathion concentrations in life-cycle exposures

Nominal concentration (μg/L)	Number of samples	Mean and standard deviation	Range
		ENDRIN	
0.40	21	0.30 ± 0.03	0.35 – 0.25
0.30	18	0.22 ± 0.02	0.26 – 0.18
0.23	18	0.18 ± 0.03	0.23 – 0.13
0.17	18	0.13 ± 0.01	0.15 – 0.11
0.13	17	0.097 ± 0.017	0.110 – 0.080
0.095	18	0.068 ± 0.010	0.087 – 0.050
0.071	17	0.051 ± 0.009	0.064 – 0.037
Control	19	0	— —
		MALATHION	
36.0	19	31.5 ± 4.4	41.8 – 26.7
27.0	19	24.7 ± 3.4	30.1 – 17.9
20.3	16	19.3 ± 1.6	22.0 – 17.7
15.2	17	15.0 ± 1.6	16.9 – 13.7
11.5	16	10.9 ± 0.8	12.2 – 9.9
8.5	17	8.6 ± 1.0	10.9 – 7.4
6.4	14	5.8 ± 0.6	6.7 – 5.1
Control	20	0	— —

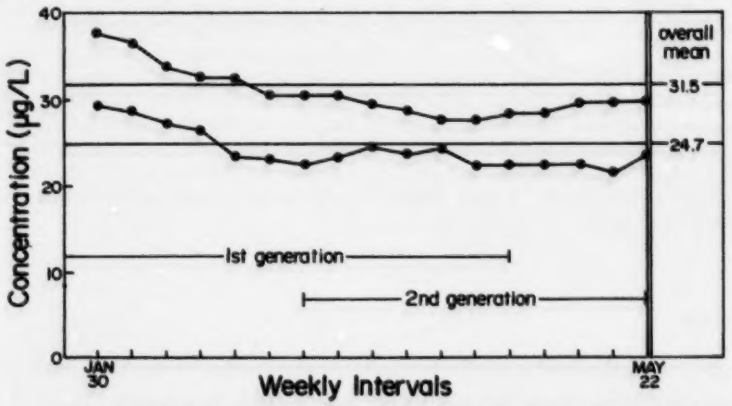

Fig 1. 4-week moving average of the two highest malathion concentrations in the life-cycle exposure. Each point represents the mean of that weekly measurement and the three previous weekly measurements. 2nd generation embryo and larvae groups were reared at various times during the 10 week interval

sampling from those fish that displayed secondary sex characteristics. At that time two spawning substrates composed of yarn-covered stainless steel screen (Smith 1973) were placed in each spawning chamber. Excess fish were held in corresponding larvae chambers until the 65th day when a final 2:5 sex ratio was established. This procedure corrected initial misidentification of immature males as mature females.

Throughout the spawning period each female was included in the determination of the mean number of eggs per female until she was removed for sex-ratio adjustment or until she died. A 64-day spawning period was allowed. Substrates were periodically removed for 2- or 3-day intervals, so the actual time the substrates were available for spawning was 54 days. Flagfish spawn on vegetation in their natural habitat (Breder and Rosen 1966), and any algae growing on the chamber surfaces could have become a spawning substrate. Therefore, the walls and bottom were scrubbed at least once each week to discourage the fish from spawning in areas other than the yarn-covered substrates. Embryos were collected from substrates every 24 hr, and samples were incubated in oscillating cups (Mount 1968) in the test water to determine viability and to provide larvae for the second-generation studies. Parental-fish studies were terminated after 110 days.

Four to seven groups of twenty 1-day-old second-generation larvae were reared for 30 days in each concentration to determine survival and growth. Total lengths and weights of each surviving fish were recorded.

In the endrin test, samples of parental fish were analyzed for whole-body endrin residues at 30, 65, and 110 days, and all surviving progeny were analyzed in the same manner at 30 days. The fish were killed by immersing them in ice cold water, and they were then frozen for later analysis. Residues were extracted and analyzed by gas chromatography according to Food and Drug Administration procedures (U.S. Department of Health, Education and Welfare 1972). Lipid contents were not measured.

All fish were fed three times each day. Fish less than 30 days old were fed live brine shrimp nauplii with occasional supplemental feedings of laboratory-cultured freshwater zooplankton and a commercial trout starter; older fish were fed frozen adult brine shrimp.

Acute exposures

The chronic-exposure systems were also used to expose 33-day-old flagfish for 216 hr to determine acute toxicity. Forty fish were randomly placed in each 90x30x30 cm test chamber of each system. Test chambers were not duplicated and each contained about 54 L of test water. Daily temperatures ranged between 24.4° and 25.2° C in both systems. Dissolved oxygen ranged from 95% to 102% saturation. Alkalinity, acidity, hardness, and pH of the test water were essentially the same as in the chronic-exposure water. Flow rates were about 10 chamber volumes/24 hr, and the photoperiod was 16 hr. Test concentrations were measured daily in all chambers that had surviving fish and the mean measured water concentrations in μg/L and standard deviations for endrin were: 3.2 ± 0.1, 2.4 ± 0.2, 1.7 ± 0.1, 1.3 ± 0.1, 1.02 ± 0.08, 0.75 ± 0.08, and 0.6 ± 0.1; and for malathion were: 516 ± 32, 374 ± 36, 294 ± 9, 233 ± 20, 170 ± 20, and 116 ± 12. Both systems had control chambers which were free of detectable insecticide residues.

Statistical analysis

Lengths, weights, and mean number of eggs per female in the chronic exposures were transformed to logarithms, and survival data were transformed to arcsin $\sqrt{\%\ \text{survival}}$. All transformed data were subjected to a one-way analysis of variance ($P = 0.05$). The endrin accumulation factors determined for both generations at 30 days were compared by a t-test. Acute LC50's were determined by probit analysis (Finney 1971).

Results

Endrin

Life-cycle exposure

Adverse effects on the parental fish, indicated by reduced growth during the first 30 days and reduced mean number of eggs produced per female, occurred only in 0.3 μg/L, the highest concentration tested (Table 2). Adverse effects on survival were not detected in any of the concentrations during any exposure intervals. However, near the end of the 2nd week some fish in 0.3 μg/L swam in whirling patterns when disturbed; a characteristic described by Mount (1962) and Johnson (1967) as one of the symptoms displayed by fish exposed to lethal endrin concentrations. After two weeks the symptoms ceased and swimming behavior appeared normal. Second-generation survival through hatching and subsequent survival and growth through the 30-day exposure were not significantly different from the control in any of the concentrations tested.

The amount of endrin accumulated per gram of fish was directly proportional to the water concentration. Therefore, the accumulation factor (concentration per gram of fish/concentration per gram of exposure water) was constant for all water concentrations, but it did change through the life cycle of the fish (Table 3).

Acute exposure

Only one fish (2.5%) died in the lowest concentration (0.6 μg/L) within 96 hr. By 216 hr 33 of the 40 (82.5%) in that same concentration were dead, and the remaining seven had such severe poisoning symptoms that they probably would have died if the experiment had continued much longer. The few surviving fish in some of the higher concentrations were also near death at the end of the test. No deaths occurred in the control during this time. The 96-hr LC50 and 95% confidence interval was calculated at 0.85 ± 0.05 μg/L. A 216-hr LC50 could not be determined because of the high mortality in all concentrations at that time.

Malathion

Life-cycle exposure

First-generation growth was significantly reduced ($P = 0.05$) at 10.9 μg/L and above during the first 30 days (Table 4). Survival during the same period was significantly reduced ($P = 0.05$) in 31.5 and 24.7 μg/L (Table 4). However, no additional adverse survival effects were detected in any of the concentrations during the 30 to 65-day and 65 to 110-day intervals. Also, the mean number of eggs produced per female was not affected by any of the concentrations. None of the seven test concentrations had any adverse effects on survival through hatching and subsequent survival and growth of the second generation.

Table 2. Survival, growth, and egg production of flagfish at life-cycle exposure to various endrin concentrations

Item	Measured concentration (μg/L)															
	0.30		0.22		0.18		0.13		0.097		0.068		0.051		Control	
	Upper chamber	Lower chamber	Upper chamber	Lower chamber	Upper chamber	Lower chamber	Upper chamber	Lower chamber	Upper chamber	Lower chamber	Upper chamber	Lower chamber	Upper chamber	Lower chamber	Upper chamber	Lower chamber
First generation																
First 30 days																
% survival	88	90	95	93	90	93	98	98	100	98	98	98	100	95	95	98
Mean length (mm)	14.7*	14.8*	15.8	16.2	16.5	15.0	16.2	16.1	16.1	16.0	16.8	16.2	16.2	16.4	16.2	15.9
Mean eggs spawned per female	191*	117*	718	269	476	782	913	696	692	1,002	746	769	1,094	780	891	574
Second generation																
30 days post-hatch																
% survival	93	43	40	90	78	83	85	73	48	90	63	98	60	90	90	96
Mean total length (mm)	24.2	21.5	22.4	22.6	22.4	23.9	21.1	24.2	23.8	21.9	23.6	23.9	22.8	22.9	22.4	22.7

* Values are significantly different from control values according to Dunnett's procedure (P = 0.05)

Table 3. Endrin accumulation factors based on whole-body wet weight for various life stages of flagfish

Generation	Number of days exposed	Number of fish per sample	Number of samples	Life stage	Accumulation factor: Mean ± standard deviation	Accumulation factor: Range
First	30	20–27	14	larva—juvenile	9,100[a] ± 2,200	5,200–12,400
	65	4–9	14	juvenile—adult	15,000 ± 1,800	11,700–18,200
	110	6–7	14	adult	7,100 ± 4,500	360–13,400
Second	30	1–20	29	larva—juvenile	12,000[a] ± 1,400	9,300–14,000

[a] The accumulation factor difference between the two generations at 30 days was significant ($P = 0.05$)

Acute exposure

At the test termination (216 hr) the few surviving fish in 516 and 374 μg/L were inactive and had developed extreme scoliosis. Fish activity increased and the frequency and severity of scoliosis decreased with decreasing concentrations. Ten percent of the fish died in 116 μg/L (lowest concentration) during this time, but scoliosis was not evident in any fish and the behavior of all surviving fish appeared normal. No fish died or developed scoliosis in the control during the experiment. The 96-hr LC50 was calculated at 349 μg/L with a 95% confidence interval at 383 to 321 μg/L and the 216-hr LC50 and 95% confidence interval was calculated at 235 ± 22 μg/L.

Discussion

Endrin

The chronic toxic concentration of 0.3 μg/L for flagfish as indicated by reduced spawning and reduced growth was similar to that found by Mount (1962) for bluntnose minnows and guppies. Johnson (1967) found adverse survival and spawning effects in adult medaka in concentrations above 0.3 μg/L. However, he found evidence of endrin toxicity to medaka larvae that were reared in control water but produced by parents exposed to concentrations less than 0.05 μg/L and concluded that the females transferred endrin to the eggs in quantities large enough to be toxic to some of the hatching larvae, especially those produced in the earlier spawnings. A transfer of toxic concentrations by the females was not evident in the flagfish experiment because the second-generation larvae in 0.3 μg/L did not exhibit any of the endrin-poisoning symptoms that were so noticeable in the early development of the first generation in the same concentration.

The absence of second-generation effects in 0.3 μg/L may have been partially due to biological variability. Another explanation is that acclimation may have occurred in the second-generation embryos, whereas the first generation was never exposed during the embryonic stage. Spehar (1976) found that flagfish larvae were more resistant to zinc if they were also exposed as embryos.

Table 4. Survival, growth, and egg production of flagfish at life-cycle exposure to various malathion concentrations

	Measured concentration															
	31.5 μg/L		24.7 μg/L		19.3 μg/L		15.0 μg/L		10.9 μg/L		8.6 μg/L		5.8 μg/L		Control	
Item	Upper chamber	Lower chamber	Upper chamber	Lower chamber	Upper chamber	Lower chamber	Upper chamber	Lower chamber	Upper chamber	Lower chamber	Upper chamber	Lower chamber	Upper chamber	Lower chamber	Upper chamber	Lower chamber
First generation																
First 30 days																
% survival	65[a]	65[a]	58[a]	75[a]	88	80	90	90	95	95	98	85	100	95	100	93
Mean total length (mm)	13.2[a]	13.1[a]	14.6[a]	13.0[a]	14.2	14.1	13.9[a]	14.3[a]	14.3[a]	14.4[a]	15.1	15.2	15.3	14.3	16.0	16.1
Mean eggs spawned per female	501	838	702	463	439	974	790	714	1,091	668	899	210	720	959	772	787
Second generation																
30 days post-hatch																
% survival	60	22	62	100	88	90	20	83	73	88	80	98	98	100	93	68
Mean total length (mm)	20.6	24.0	19.8	21.7	20.2	21.2	22.5	21.2	21.6	22.5	21.9	22.7	22.3	20.5	20.7	22.4

[a] Values are significantly different from control values according to Dunnett's procedure (P = 0.05)

Although lipids were not measured, it is likely that the change in endrin-residue levels in the tissues was directly related to the lipid content. Jarvinen *et al.* (in preparation) found that the reduction of DDT residues in fathead minnows during spawning corresponded to the reduction of lipids. The same pattern appeared in the flagfish experiment: Residues were lower after the 64-day spawning period (110 days of exposure). The significantly higher accumulation factor of the second-generation fish at 30 days may have been due to higher lipid content resulting in storage of more lipid soluble endrin. These fish were about 40% longer than the first-generation fish at 30 days, which was probably brought about by less crowded conditions and more available food. The second-generation studies began with 20 fish per chamber, whereas the first-generation studies started with 40 fish per chamber.

Flagfish spawn intermittently throughout the daylight hours (Martz and Barlow 1966, Smith 1973). For this reason the number of spawns per female and the number of eggs per spawn could not be determined. The only measure of fecundity was the mean number of eggs per female during the 64-day spawning period. Even this measurement had its limitations because the females were constantly attempting to feed on the eggs they spawned, and the defending male was not always able to protect the eggs, especially when two or more females invaded his territory at the same time. This predation was observed in all spawning chambers, including the controls. The male territorial defense appeared to be as rigorous in the 0.3 μg/L chambers as in the control chambers. Therefore, the collection of significantly fewer eggs in 0.3 μg/L was believed to be due to reduced spawning rather than increased predation.

Males discontinued their territoriality and "schooled" with the females when spawning substrates were removed for 2- to 3-day periods. Spawning was never observed in the absence of spawning substrates.

Malathion

Effects on growth of the first generation during the first 30 days were measurable at 10.9 μg/L and above. How these subtle growth effects might affect a natural population is difficult to estimate, but the reduced growth and the sharp decrease in survival in 24.7 and 31.5 μg/L might limit the number of fish reaching maturity to such an extent that reproduction would be insufficient to maintain the population.

Chronic toxic concentrations for both fathead minnows (Mount and Stephan 1967) and bluegills (Eaton 1970) were determined partially or fully from spinal deformities, but the flagfish chronic effects in this study were based on reduced growth and reduced survival in the first generation during the first 30 days. No evidence of deformities was found in the flagfish at the chronic concentrations tested. However, scoliosis developed in the fish in the acute test within 216 hr when exposed to concentrations above 116 μg/L.

No discernible adverse effects occurred in the second generation in any of the concentrations tested. The two highest concentrations decreased with time; means of these two concentrations during the first 30 days of the first-generation exposure were about 20% to 40% higher than their means during the 30-day exposure periods of the second generation (Figure 1). However, this shift in

concentrations could not be the reason for the lack of effects in all the concentrations. Post-hatch survival in 31.5 μg/L appeared to be lower than in the other concentrations, but the variability between batches within concentrations was too high to show any statistical significance. Explanations for the absence of second-generation reduced growth in the higher concentrations may be the same as those given for the endrin test.

Acknowledgments. I thank Miss H. Herrmann for assisting in the daily operations of the tests, Messrs. L. Mueller and C. Anderson and Mrs. M. Hoffman for the water and tissue-residue analysis, and other members of the Environmental Research Laboratory-Duluth for advice and critical review of the manuscript.

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Manuscript received March 8, 1977; accepted October 19, 1977